

Supplementary Data

Chlorogenic acid inhibits epithelial-mesenchymal transition and invasion of breast cancer by down-regulating LRP6

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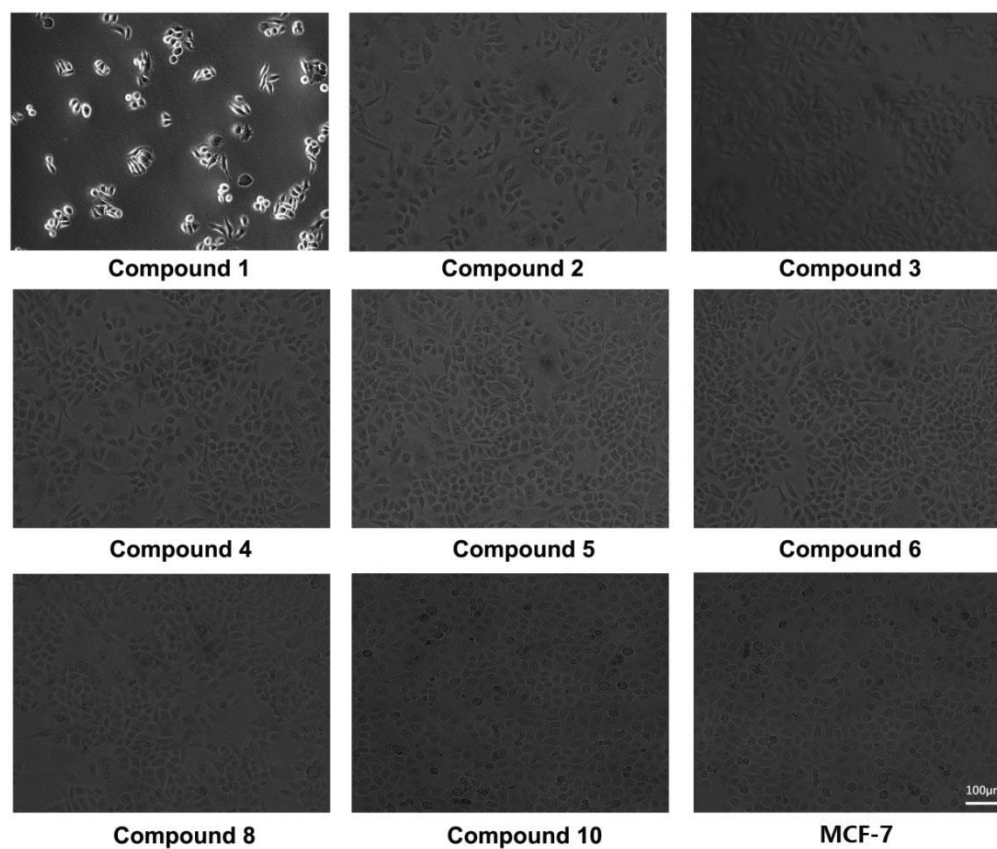
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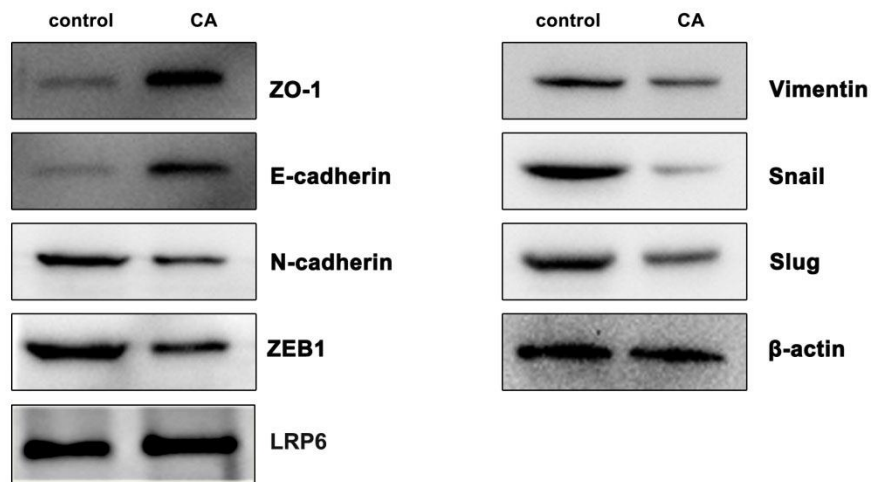
Supp. Fig. 1

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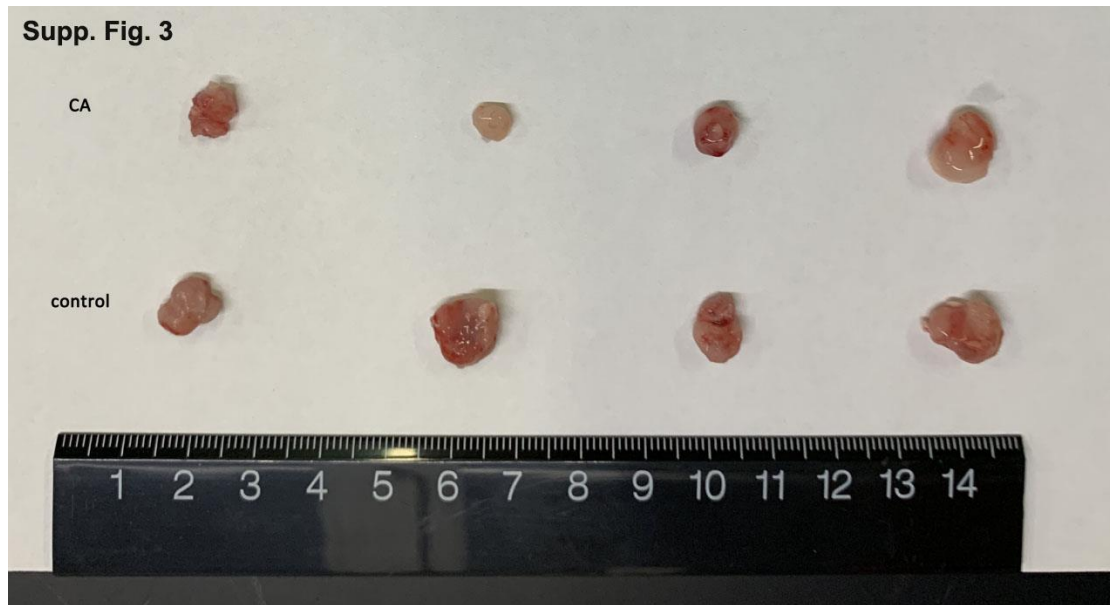


Supplemental figure 1. After 48 h treatment with 6.25 μ M small molecules, the morphology of MCF-7 cells was observed using a microscope (200 \times magnification; scale bar = 100 μ m).

Supp. Fig. 2

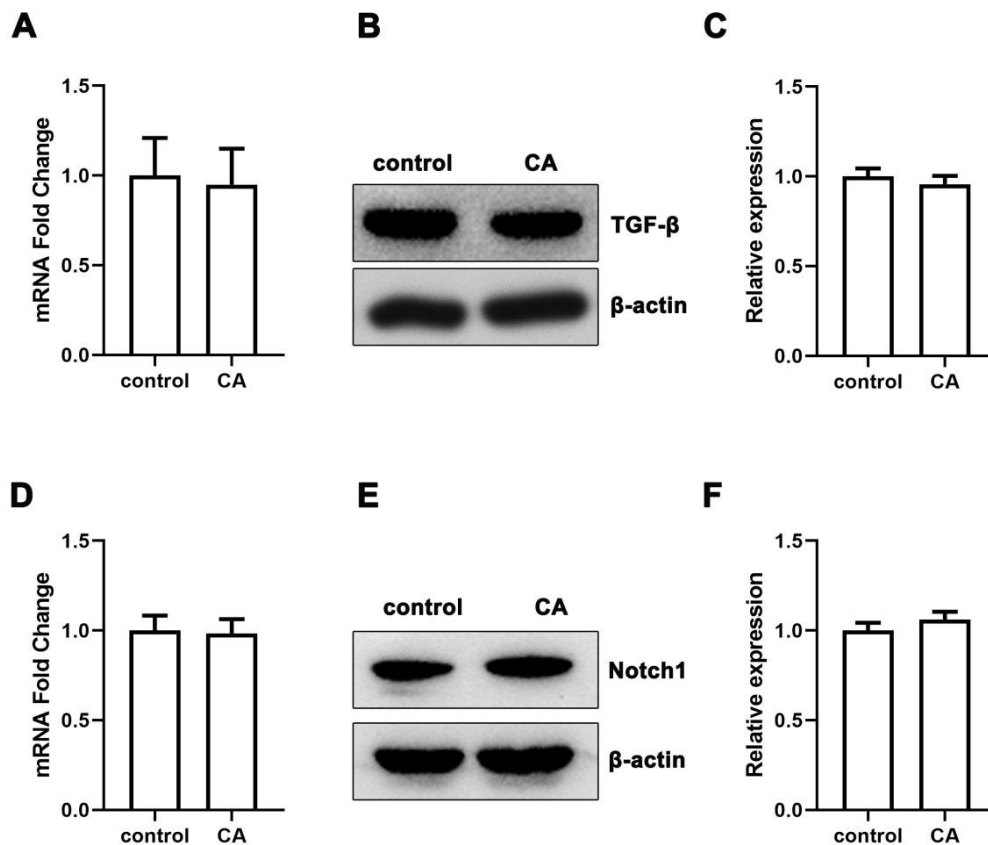


Supplemental figure 2. CA inhibited EMT in MDA-MB-231 cells. The expression of epithelial markers (ZO-1 and E-cadherin) and mesenchymal markers (N-cadherin, ZEB1, Vimentin, Snail, and Slug) was evaluated by Western blot experiment.



Supplemental figure 3. CA inhibited tumor growth in nude mouse xenograft model. Tumor tissue of mouse treated with CA was taken, and model group was the control group.

Supp. Fig. 4



Supplemental figure 4. CA had no effect on TGF-β signaling and Notch1 signaling in MCF-7 cells. (A) The mRNA level of TGF-β was determined by qPCR assay. (B) The protein level of TGF-β was determined by Western blot assay. (C) Relative expression of TGF-β was quantified. (D) The mRNA level of Notch1 was determined by qPCR assay. (E) The protein level of Notch1 was determined by Western blot assay. (F) Relative expression of Notch1 was quantified. β-actin was considered as an internal control. Data are shown as the mean \pm SD (n=3). * P <0.05 and ** P <0.01 versus the control group.

Supplemental table 1. Primers for qPCR assay.

Gene	Forward (5'-3')	Reverse (5'-3')
ZO-1	GTGTTGTGGATACCTTGT	GATGATGCCTCGTTCTAC
E-cadherin	GGACTTTGGCGTGGGCCAGG	CCCTGTCCAGCTCAGCCCGA
N-cadherin	ACAGTGGCCACCTACAAAGG	CCGAGATGGGGTTGATAATG
ZEB1	GCCAATAAGCAAACGATTCTG	TTTGGCTGGATCACTTTCAAG
Vimentin	GAGAACTTTGCCGTTGAAGC	CTCAATGTCAAGGGCCATCT
Snail	CTGCGGGAAGGCCTTCTCT	CGCCTGGCACTGGTACTTCTT
Slug	TGTGTGGACTACCGCTGC	TCCGGAAAGAGGAGAGAGG
LRP6	GATTATCCAGAAGGCATGGCAG	TCCCATCACCATCTTCCA
GAPDH	GGCCTCCAAGGAGTAAGACC	AGGGGTCTACATGGCAACTG

Supplemental table 2. The viability of MCF-7 cells treated by the selected 10 small molecules.

Concentration (μ M)	Control (%)	#1 (%)	#2 (%)	#3 (%)	#4 (%)	#5 (%)	#6 (%)	#7 (%)	#8 (%)	#10 (%)
25	100	76.71	41.81	1.99	93.92	38.40	60.66	76.27	36.09	83.57
12.5	100	91.56	59.23	16.32	93.90	49.90	65.35	83.29	55.22	97.84
6.25	100	90.84	81.14	104.24	95.84	76.62	78.61	86.63	78.50	91.37